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FORM PTO-1390 (REV. 9-2001) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: BSWV-P01-002

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 10/018127

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU00/00643	07 June 2000	07 June 1999

TITLE OF INVENTION: A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS

APPLICANT(S) FOR DO/EO/US: CLANCY, Robert Llewellyn; GLEESON, Maree

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [X] has been previously submitted ~~under 35 U.S.C. 154(d)(4)~~.
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [X] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). **(1 p., unsigned)**
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [X] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information:

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/018127	PCT/AU00/00643	BSWV-P01-002

21. [X] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 740	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	36 - 20 =	16	x $18.00	$ 288
Independent claims	3 - 3 =	0	x $84.00	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$ 280

TOTAL OF ABOVE CALCULATIONS =	$1,308
[X] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +	$
SUBTOTAL =	$ 654
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$
TOTAL NATIONAL FEE =	$ 654
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +	$
TOTAL FEES ENCLOSED =	$
Amount to be refunded:	$
charged:	$ 654

a. [ ] A check in the amount of $ _____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 18-1945 in the amount of $ 654 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 18-1945 . A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

PATENT GROUP
Ropes & Gray
One International Place
Boston, MA 02110
U.S.A.

SIGNATURE

Matthew P. Vincent
NAME

36,709
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Robert Llewellyn Clancy, Maree Gleeson.	:	
		:Docket No.:	BSWV-P01-002
Appln. No.	35 USC 371 of PCT/AU00/00643	:	:
International Filing Date:	07 June 2000	:	

For: A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS

BOX PCT (DO/EO/US)
Assistant Commissioner for Patents
Washington, D.C. 20231

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7 December 2001
Date of Signature and of Mail Deposit

Michael Crowley
Printed name: Michael Crowley

PRELIMINARY AMENDMENT

Dear Sir:

Preliminary to examination on its merits, kindly amend the above-referenced patent application as follows:

In the Specification:

Page 1, line 1, after the title and before "Technical Field" please insert:

--This application is the U.S. national phase application of, and claims priority from, PCT/AU00/00643, international filing date 07 June 2000, and from Australia PQ 0810, filed 07 June 1999, the specifications of which are incorporated herein by reference.--

In the claims:

Please cancel existing claims 1-19 and add the following new claims:

--20. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

(a) determination of the immunoglobulin A (IgA) level in a sample from the subject; and

(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.

21. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

(a) determination of immunoglobulin A1 (IgA1) level in a sample from the subject; and

(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.

22. A method according to claim 20 or claim 21 wherein the subject is a human infant.

23. A method according to claim 20 or claim 21 wherein the sample is a sample from a subject at the time of, or any time up to approximately 2 weeks after, an upper respiratory tract infection (URTI) and/or symptoms.

24. A method according to claim 20 or claim 21 wherein the immunoglobulin is secretory immunoglobulin.

25. A method according to claim 20 or claim 21 wherein the immunoglobulin is salivary immunoglobulin.

26. A method according to claim 20 or claim 21 wherein the sample is whole unstimulated saliva.

27. A method according to claim 20 or claim 21 wherein the subject is not fasting when the sample is collected.

28. A method according to claim 20 or claim 21 wherein the immunoglobulin level is determined by ELISA.

29. A method according to claim 20 or claim 21 wherein the immunoglobulin level is determined by radial immunodiffusion.

30. A method according to claim 20 or claim 21 wherein the immunoglobulin level is analysed by a rapid near-subject assay.

31. A method according to claim 20 or claim 21 wherein the immunoglobulin level is determined by contacting a body secretion with an assay device or system on a support.

32. A method according to claim 20 or claim 21 wherein the immunoglobulin level is analysed by contacting an assay device or system with the saliva of the subject *in situ*.

33. A method according to claim 20 or claim 21 wherein the standard is a normal population standard.

34. A method according to claim 20 or claim 21 wherein the standard is an internal personal standard.

35. A method according to claim 20 or claim 21 further including comparison of the ratio of immunoglobulin level to other indices.

36. A method according to claim 20 or claim 21 further including comparison of the ratio of immunoglobulin level to other indices selected from the group consisting of IgM, IgG, acute phase reactants and other cellular components.

37. A method for assessing potential susceptibility to development of ALTE and/or SIDS in an infant including:

(a) determination of the immunoglobulin A (IgA) and/or immunoglobulin A1 (IgA1) level in a sample of the infant's whole, unstimulated saliva; and

(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA and/or said IgA1 level with a predetermined standard.

38. A kit when used in a method according to any one of claims 20, 21, or 37. --

REMARKS

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Applicants hereby request that any fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945.**

Respectfully Submitted,

Date: 07 December 2001

Customer No: 28120
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PCT/AU00/00643

A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS

TECHNICAL FIELD

The present invention relates to methods for determining predisposition to acute life threatening episodes (ALTE) and/or sudden infant death syndrome (SIDS) and in particular to methods of assessing potential susceptibility to development of ALTE and/or SIDS by determining a subject's total IgA and/or IgA1.

BACKGROUND

A great deal has been done to minimize the risk of SIDS by non-specific methods related to infant care. However, prevention using specific assays related to causal mechanisms has not been explored. Identifying a causal mechanism may be expected to make a major impact on SIDS outcome through general awareness, and if used in conjunction with non-specific nursing care. The development of new techniques for identifying infants at risk of SIDS could be a significant outcome.

Interest in this approach to the prevention of SIDS arose as a result of an unusual opportunity of observing a 'prospective' case of SIDS during a study of 250 normal infants [1]. The infants were followed from birth, measuring parameters of immune status in saliva. The key observation in the one child who died from SIDS was an extraordinarily high IgM level appearing after a mild respiratory tract infection, several weeks before the child suddenly died. While all parameters tested in the SIDS victim (i.e. albumin, IgG and IgA) were in excess of the 90th percentile level, relative levels of IgM were the highest, being more than three times the level of the 90th percentile figure (compared to approximately one and a half times the 90th percentile level for albumin, IgG and IgA). This observation in a single case was consistent with post mortem studies

showing large numbers of IgM containing plasma cells in the trachea and gut of subjects dying from SIDS [2-5]. The level of IgM was much in excess of any small increases seen in matched infection control studies [6]. These observations raised the possibility that assay of IgM in saliva of infants with an upper respiratory tract infection may be a very useful marker of risk of SIDS, reflecting the disturbed mucosal immunoregulation that underpins the risk.

The numerous epidemiological studies of SIDS have identified many of the risk factors of SIDS but have failed to find a cause [7]. The role of infection and disturbed immunity has been proposed as one of the potential mechanisms for SIDS [8]. The common findings at autopsy of SIDS infants are consistent with infection or inflammation as a contributing cause of death [9]. SIDS has been reported to occur after a mild upper respiratory tract infection (URTI) [9-12], however there is no evidence that favours infections by any virulent pathogen. A low grade pathogen, that results in overstimulation of the immune system may be one important link in the chain of events that culminates in respiratory arrest.

There is evidence from post mortem studies [2-5, 13-14] and a prospective case study [1] of a gross disturbance of mucosal immunity in SIDS associated with prior respiratory illness or inflammation. These studies suggest an infective agent is responsible for the disturbance observed in the immune parameters which thus provides a clinical "trigger" for testing the infant for risk..

Infants presenting with episodes of apnoea from which the infant recovers are termed acute life threatening episodes (ALTEs) and are classified as "near-miss" SIDS when no underlying medical condition is identified. ALTE children could, therefore, be

expected to have a similar pattern of dysregulation of mucosal immunity to SIDS children.

To date, however, no method exists by which a prediction of the potential susceptibility to development of ALTE and/or SIDS can be carried out on the basis of a specific immunological assay.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

SUMMARY OF THE INVENTION

As indicated above, the prior art [1] pointed towards IgM levels after URTI infection as being a potentially useful parameter for study in ALTE/SIDS research. However, it has been unexpectedly found that IgA levels were significantly and consistently higher in ALTE or "near miss" SIDS cases. IgA can therefore be used as a predictor of susceptibility to development of ALTE and/or SIDS.

According to a first aspect, the present invention provides a method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

a) determination of the immuoglogulin A (IgA) level in a sample from the subject; and

b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.

According to a second aspect, the present invention provides a method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

a) determination of immunoglobulin A1 (IgA1) level in a sample from the subject; and

b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.

Preferably, the subject is a human infant.

Preferably, the sample is a sample from a subject at the time of, or any time up to approximately 2 weeks after, an upper respiratory tract infection (URTI) or symptoms.

Preferably, the immunoglobulin is secretory immunoglobulin. Preferably, the secretory immunoglobulin is salivary immunoglobulin. Preferably, the sample is whole unstimulated saliva. However, it will be clear to the skilled addressee that other body secretions known to contain IgA and/or IgA1 would also be useful as samples for the present method.

Preferably, the subject is not fasting when the sample is collected.

Preferably, the immunoglobulin level is determined by ELISA. However, it will be understood that the immunoglobulin level may be determined by radial immunodiffusion and/or similar methods, all of which would be known to a skilled addressee. The method is particularly suitable for an assay in which the immunoglobulin level is analysed by a rapid, near-subject assay. It can thus provide a yes/no test for immediate action.

In one embodiment, the immunoglobulin level is determined by contacting a body secretion with an assay device or system on a support. The sample need not necessarily be removed from the subject but the method may be applied *in situ*. For example, the immunoglobulin level may be analysed by contacting an assay device or system with the saliva of the subject *in situ*.

The predetermined standard may be any appropriate standard, for example, a normal population standard or an internal personal standard. The skilled addressee will recognize the types of standards which will be useful in the present invention.

In a third aspect, the present invention provides a method for assessing potential susceptibility to development of ALTE and/or SIDS in an infant including:

(a) determination of the immunoglobulin A (IgA) and/or immunoglobulin A1 (IgA1) level in a sample of the infant's whole, unstimulated saliva; and

(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA and/or said IgA1 level with a predetermined standard.

It will be clear to the skilled addressee that the determination of the level of IgA or IgA1 could also be used as a predictor of susceptibility to development of ALTE and/or SIDS in conjunction with other indices such as other immunoglobulins, for example IgM or IgG, acute phase reactants or cellular components.

In a fourth aspect, the present invention provides a kit when used in a method according to any one of the first to third aspects.

In the context of the present invention, the word "standard" includes within its meaning, but is not limited to, the normal population level of immunoglobulin ie. the average IgA or IgA1 value for age-matched normal subjects. It may also be an internal personal standard i.e. the level of IgA or IgA1 in a sample taken previously from the same individual.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 IgA concentration levels (mg/L) for ALTE (■), "mild" (O) and well (▲) infants - initial sample.

Figure 2 IgM concentration levels (mg/L) for ALTE (■), "mild" (O) and well (▲) infants - initial sample.

Figure 3 IgG concentration levels (mg/L) for ALTE (■), "mild" (O) and well (▲) infants - initial sample.

Figure 4 IgA concentration levels (mg/L) for ALTE (■), "mild" (O) and well (▲) infants - 14 day sample. Subject RO3 was assessed 12 days post infection.

Figure 5 IgM concentration levels (mg/L) for ALTE (■), "mild" (O) and well (▲) infants - 14 day sample. Subject RO9 was assessed 14 days post immunisation with triple antigen and *Haemophilus influenzae* B.

Figure 6 IgG concentration levels (mg/L) for ALTE (■), "mild" (O) and well (▲) infants - 14 day sample.

Figure 7A IgA1 concentration levels (mg/L) for ALTE, "mild" and well infants.

Figure 7B Data of Figure 7A shown with 95% confidence intervals.

Figure 8 Total IgA concentration levels (mg/L) for ALTE, "mild" and well infants in which IgA1 concentration levels (mg/L) is shown in Figures 7A and 7B.

DESCRIPTION OF THE INVENTION

A preferred embodiment of the invention will now be described, by way of example only.

EXAMPLE 1

Total IgA, IgG and IgM levels in Saliva of Infants with ALTE

Saliva Collection

Whole mixed saliva was collected by gentle suction from the buccal cavity of the mouth [15]. This technique is successful in children (aged from 1 day) and adults [1,16].

Questionnaire

A standardised questionnaire was used to collect the relevant SIDS related demographics. The classification into the "near-miss" SIDS group (ALTE) was made by the attending paediatrician on the basis of clinical investigations.

Saliva Tests

Salivary immunoglobulins were measured by ELISA and albumin by rate nephelometry (Beckman, ARRAY) [16].

Statistical Analysis

The differences in mucosal immune parameters was determined between the ALTE infants and two control groups of subjects (mild URTI and well infants) using analysis of variance (ANOVA) or the appropriate non-parametric statistics.

Subjects

37 subjects aged 1-10 months were recruited (20 males, 17 female) in 3 categories:

- Acute life Threatening episodes (ALTE) at John Hunter Hospital (n=5)
- Mild respiratory tract illness (MILD) from General Practitioners (n=11)
- A well control group (WELL) from immunisation clinics (n=21).

Questionnaire Data

- There were more males (n=4) than females (n=1) in the ALTE group.
- There were no significant differences between the groups for age, birth history, family demographics, ethnic background or family history of SIDS.
- There were a higher percentage of children exposed to passive tobacco smoke (60%, n=3) in the ALTE group compared to the MILD (36%, n=4) and WELL (10%, n=2) control groups (p=0.03).

- The ALTE group had a higher percentage of families in the average-below average socio-economic category (100%) compared to the other control groups ($p<0.01$).
- There were no differences between the groups for feeding history, immunisation status, sleeping position.
- In 4 of the 5 ALTE subjects an Upper Respiratory Tract Illness (URTI) was suspected as the cause of the ALTE (Table 1).

TABLE 1

Doctor Questionnaire - ALTE

	IgA (mg/L)		Q12	Q32	Q34	Q40	Follow Up
Study Number	Initial	14 Day	Face Covered	Prior URTI	Passive Smoke	URTI Suspected	Suspected Clinical Cause
A01	115.5	56.1	N	N	Y	N	Gastro-esophageal reflux
A02	228.9	22.8	N	Y	Y	Y	RSV+ve Bronchiolitis
A03	410.6	230.9	N	Y	Y	Y	RSV+ve Bronchiolitis
A04	91.0	28.9	Y	Y	N	Y	RSV+ve Bronchiolitis
A05	26.6	79.2	N	Y	N	Y	Reflux with aspiration

Salivary Immunoglobulins

Two samples of saliva were collected from each subject. The first sample was collected from the ALTE group within 24 hours of admission to hospital and from the MILD respiratory illness group within 48 hours of presentation of their General Practitioners. The WELL group were recruited from immunisation clinics and saliva

collected at ages to approximate the ages of presentation of the ALTE and MILD groups. The second sample was collected 14 days later from each subject.

The figures in Appendix C have the age related 5th-95th percentile reference ranges indicated for each salivary immunoglobulin over the first year of life.

- The salivary IgA, IgG and IgM concentration in the ALTE group were all significantly higher than the MILD (Tables 2A and 2B) and WELL (Tables 3A and 3B) groups for both sample 1 and 2 (Figures 1 and 2).
- There were no significant differences between the MILD and WELL groups for either sample 1 or sample 2 (Tables 2C and 3C).
- There were two subjects in the MILD group who had grossly elevated salivary immunoglobulin concentrations in the 14 day collections. (See Appendix C).
  - RO3 had an elevated IgA 12 days post infection.
  - RO9 had an elevated IgM that is most likely accounted for by immunisation with Triple antigen and *Haemophilus influenzae* B 14 days prior to the saliva collection.

TABLE 2A
First Sample
Analysis of Immunoglobulins - ALTE vs MILD

	ALTE			MILD			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	115.55	(27-411)	11	9.93	(0-37)	<0.01
IgG	5	9.21	(0-16)	11	0.00	(0-3)	0.02
IgM	5	4.61	(3-24)	11	2.18	(0-16)	0.04

TABLE 2B
First Sample
Analysis of Immunoglobulins - ALTE vs WELL

	ALTE			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	115.55	(27-411)	21	11.37	(0-67)	<0.01
IgG	5	9.21	(0-16)	21	0.00	(0-8)	0.01
IgM	5	4.61	(3-24)	21	1.00	(0-33)	0.01

TABLE 2C
First Sample
Analysis of Immunoglobulins - MILD vs WELL

	MILD			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	11	9.93	(0-37)	<0.01	11.37	(0-67)	0.68
IgG	11	0.00	(0-3)	0.02	0.00	(0-8)	0.66
IgM	11	2.18	(0-16)	0.04	1.00	(0-33)	0.36

TABLE 3A
Second Sample
Analysis of Immunoglobulins - ALTE vs MILD

	ALTE			MILD			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	56.06	(23-231)	11	8.88	(1-255)	0.04
IgG	5	2.99	(2-7)	11	0.00	(0-4)	0.03
IgM	5	9.39	(2-16)	11	2.31	(0-27)	0.07

TABLE 3B Second Sample Analysis of Immunoglobulins - ALTE vs WELL							
	ALTE			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	56.06	(23-231)	20	10.53	(0-58)	<0.01
IgG	5	2.99	(2-7)	20	0.00	(0-6)	<0.01
IgM	5	9.39	(2-16)	20	1.66	(0-14)	<0.01

TABLE 3C Second Sample Analysis of Immunoglobulins - ALTE vs WELL							
	MILD			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	11	8.88	(1-255)	20	10.53	(0-58)	0.71
IgG	11	0.00	(0-4)	20	0.00	(0-6)	0.75
IgM	11	2.31	(0-27)	20	1.66	(0-14)	0.56

Conclusions

- The grossly elevated salivary IgA concentration in 4 of 5 ALTE subjects at presentation was not observed in the MILD or WELL control groups. Salivary IgA can therefore act as a marker for ALTE (and SIDS) in subjects presenting with an otherwise mild respiratory illness. This was an unexpected result since the prior art [1] suggested IgM would be the most useful parameter in prediction of ALTE/SIDS susceptibility.

- The elevated salivary IgA and IgM concentrations in 4 of 5 ALTE support the concept of an infection or inflammatory cause in ALTE (and SIDS).
- RSV positive Bronchiolitis was evident in 3 of 5 ALTE subjects.

EXAMPLE 2

Total IgA and IgA1 subclass in Saliva of Infants with ALTE

Study Groups

Saliva samples were collected from infants on the day of admission to hospital for an unexplained acute life-threatening episode (ALTE). The infants were included in this study if all congenital or obstructive causes of apnoea had been excluded. This group of subjects have been classified as the "near-miss SIDS" infants.

Saliva was collected from age matched control subjects in two categories. Normal healthy infants were recruited from the Child Immunisation Clinics and classified as WELL infants. The second group was recruited from general practitioners, who referred infants with a mild upper respiratory infection and these infants were classified as the MILD infection control group. Saliva was collected on the day of referral with the mild infection.

Laboratory Analysis

Saliva samples were assayed by an Enzyme Linked Immunosorbant Assay (ELISA) to detect total IgA and IgA1 subclass antibodies. The assay uses a WHO/IUIS approved monoclonal antibody for IgA1 subclass as the capture antibody in conjunction with a polyclonal antibody-enzyme labelled detection system.

Results

The results indicate that the concentrations of IgA1 subclass in the saliva from infants suffering an ALTE were significantly higher than the concentrations for the infants in the control groups of normal healthy infants and those suffering a mild upper respiratory infection (p=0.009) (Table 4 and Figures 7A and 7B).

The concentrations of IgA1 in saliva from the normal healthy infants were not significantly different from those with mild respiratory illnesses.

Five samples were assayed from each of the three study groups: ALTE babies, babies with mild infection, and well babies. The level of IgA1 was generally much higher in the samples from the ALTE babies compared to the levels in the other two groups (p=0.009). Levels in the mild infection and well baby groups were similar.

Statistical Analysis

The non-parametric Kruskal-Wallis test was used to compare the distributions of IgA1 values for the three groups. The probability of the three sample groups having equal IgA1 distributions is p=0.009. Due to small sample sizes, the estimated 95% confidence intervals about the group medians are equivalent to the range (ie. min, max) of the data.

Table 1

	IgA1 (mg/L)		
group	min	max	median
ALTE	12.27	96.35	68.35
Mild infection	2.79	9.78	9.92
Well	3.02	14.85	7.29

Total IgA levels were also elevated in the same infants who took part in this study (Figure 8).

Conclusion

IgA1 concentrations are significantly elevated in infants suffering an unexplained ALTE (4 out of 5 children). Three out of the same 5 children with ALTE were found to have elevated total IgA levels. Therefore, although it is clear that both IgA and IgA1 are useful parameters in the prediction of ALTE, IgA1 levels may be the more useful parameter. Since ALTE are classified as "near-miss" SIDS (when no other medical condition is identified), it follows that both IgA and IgA1 are also useful parameters in the prediction of SIDS.

Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

a) determination of the immuoglogulin A (IgA) level in a sample from the subject; and

b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.

2. A method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

a) determination of immunoglobulin A1 (IgA1) level in a sample from the subject; and

b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.

3. A method according to claim 1 or claim 2 wherein the subject is a human infant.

4. A method according to any one of claims 1 to 3 wherein the sample is a sample from a subject at the time of, or any time up to approximately 2 weeks after, an upper respiratory tract infection (URTI) and/or symptoms.

5. A method according to any one of claims 1 to 4 wherein the immunoglobulin is secretory immunoglobulin.

6. A method according to claim 5 wherein the secretory immunoglobulin is salivary immunoglobulin.

7. A method according to claim 6 wherein the sample is whole unstimulated saliva.

8. A method according to any one of claims 1 to 7 wherein the subject is not fasting when the sample is collected.

9. A method according to any one of claims 1 to 8 wherein the immunoglobulin level is determined by ELISA.

10. A method according to any one of claims 1 to 8 wherein the immunoglobulin level is determined by radial immunodiffusion.

11. A method according to any one of claims 1 to 9 wherein the immunoglobulin level is analysed by a rapid near-subject assay.

12. A method according to any one of claims 1 to 11 wherein the immunoglobulin level is determined by contacting a body secretion with an assay device or system on a support.

13. A method according to any one of claims 1 to 12 wherein the immunoglobulin level is analysed by contacting an assay device or system with the saliva of the subject *in situ*.

14. A method according to any one of claims 1 to 13 wherein the standard is a normal population standard.

15. A method according to any one of claims 1 to 13 wherein the standard is an internal personal standard.

16. A method according to any one of claims 1 to 15 further including comparision of the ratio of immunoglobulin level to other indices.

17. A method according to claim 16 wherein the other indices are selected from the group consisting of IgM, IgG, acute phase reactants or cellular components.

18. A method for assessing potential susceptibility to development of ALTE and/or SIDS in an infant including:

(a) determination of the immunoglobulin A (IgA) and/or immunoglobulin A1 (IgA1) level in a sample of the infant's whole, unstimulated saliva; and

(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA and/or said IgA1 level with a predetermined standard.

19. A kit when used in a method according to any one of claims 1 to 18.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS

(57) Abstract: The present invention relates to methods for determining predisposition to apparent life-threatening events (ALTE) (also referred to as "acute life threatening episodes") and/or sudden infant death syndrome (SIDS) and in particular to methods of assessing potential susceptibility to development of ALTE and/or SIDS by determining a subject's total IgA and/or IgA1 level.

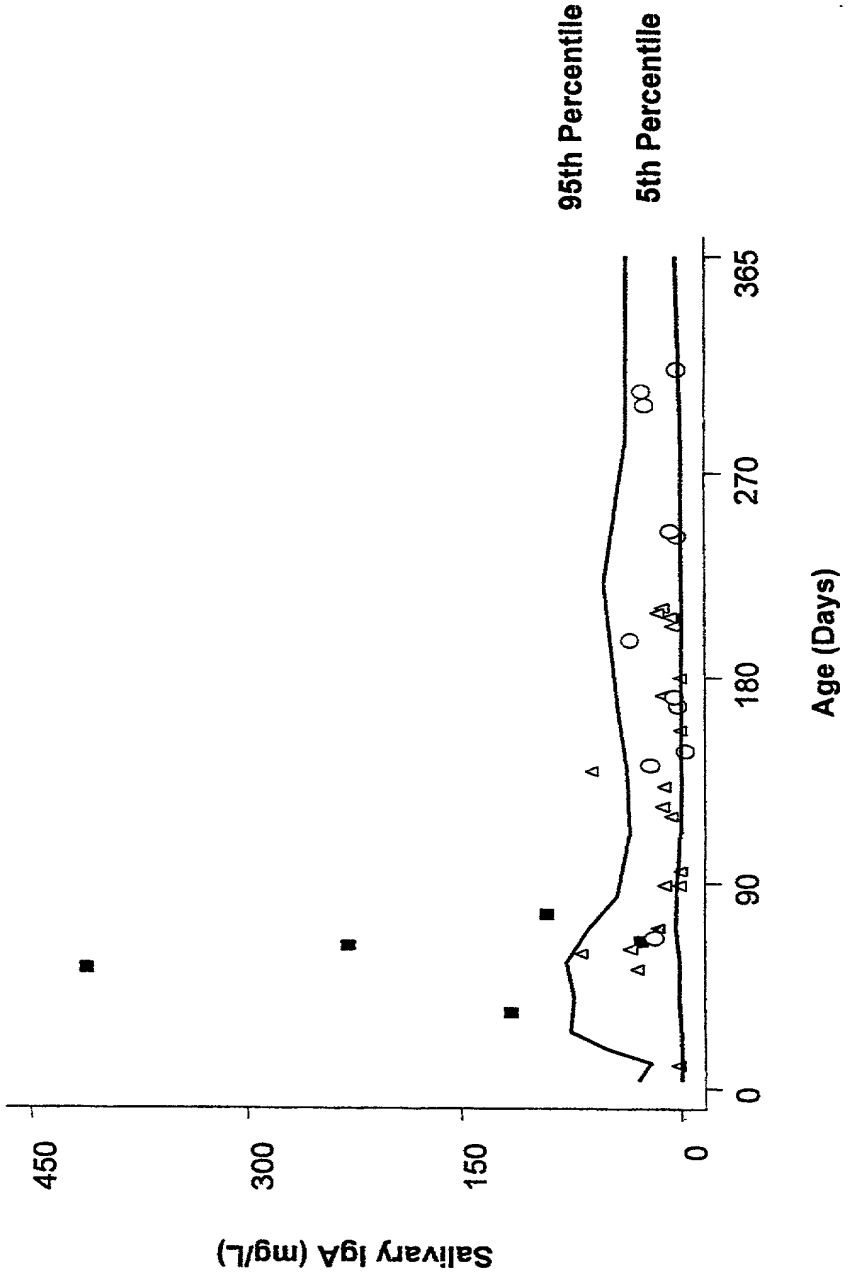

95th Percentile
5th Percentile
Salivary IgA (mg/L)
0
150
300
450
0
90
180
270
365
Age (Days)

Figure 1

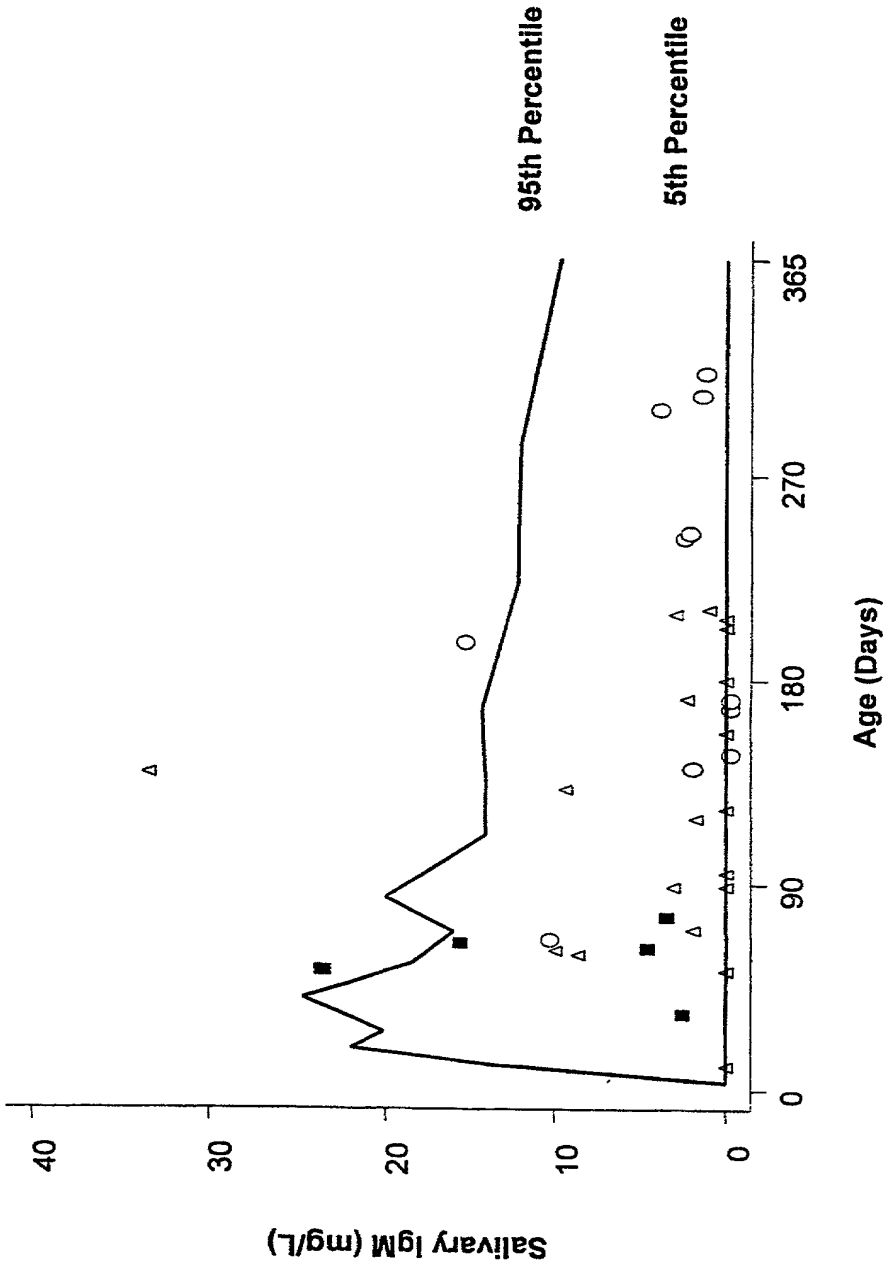
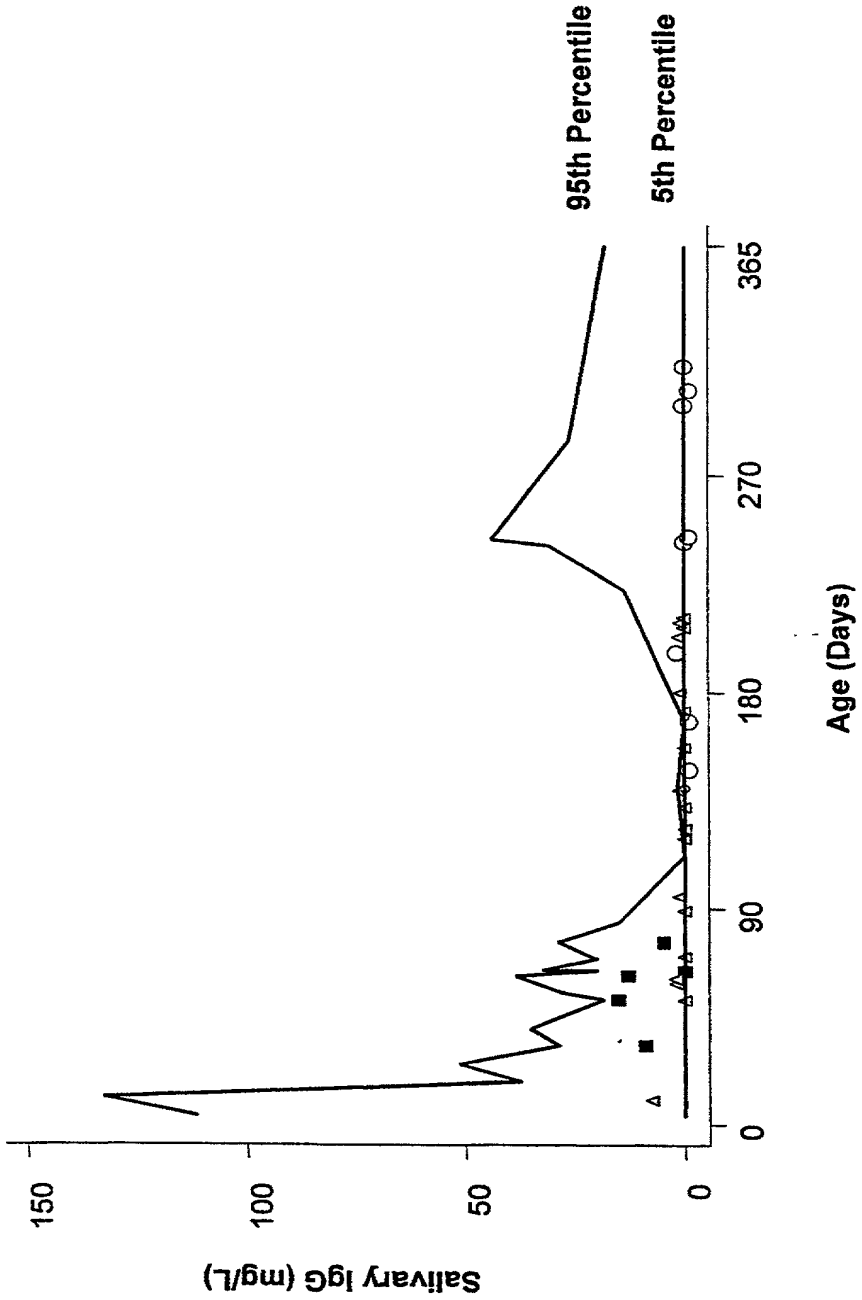

95th Percentile
5th Percentile
Salivary IgM (mg/L)
0
10
20
30
40
0
90
180
270
365
Age (Days)

Figure 2


95th Percentile
5th Percentile
Salivary IgG (mg/L)
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100
50
0
0
90
180
270
365
Age (Days)

Figure 3

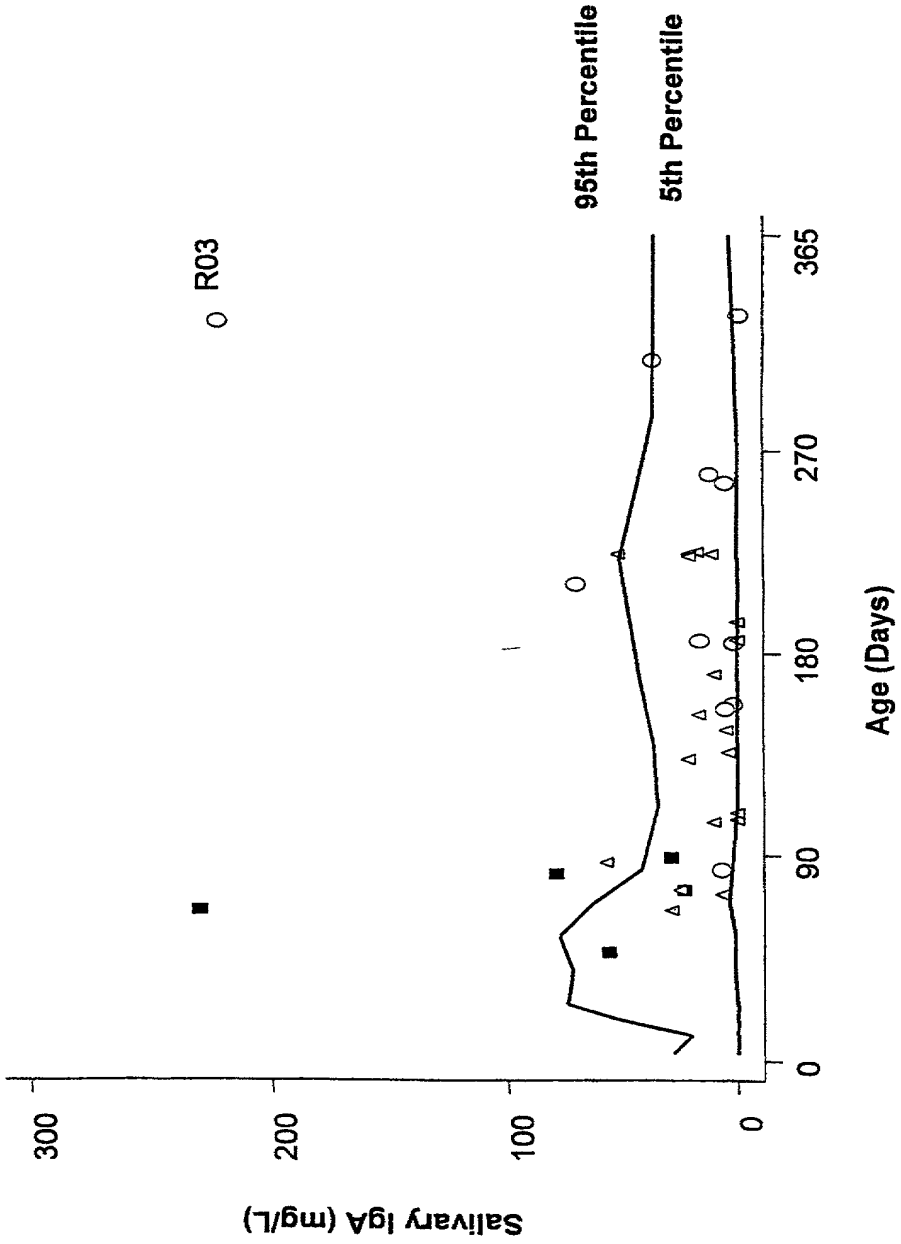


Figure 4

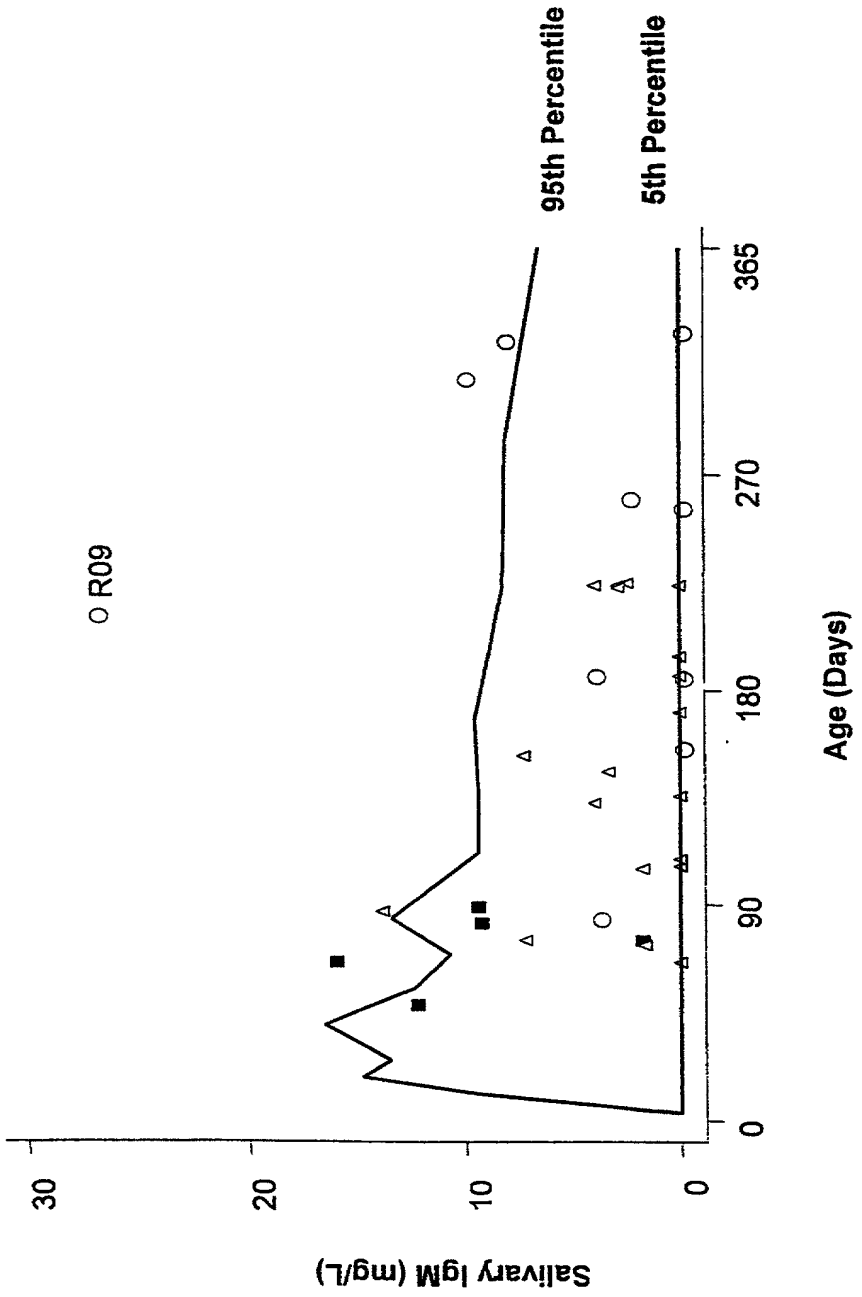

95th Percentile
5th Percentile
R09
Salivary IgM (mg/L)
Age (Days)
0
10
20
30
0
90
180
270
365

Figure 5

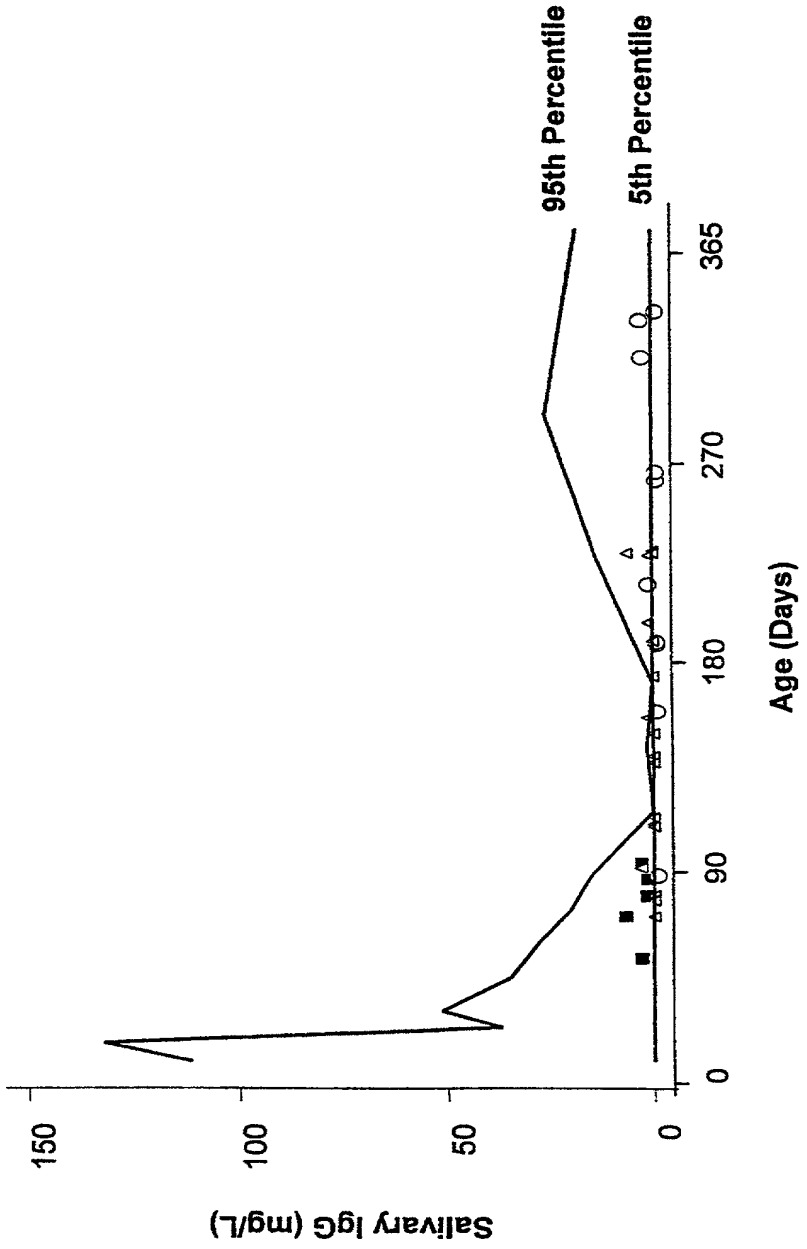

95th Percentile
5th Percentile
Salivary IgG (mg/L)
150
100
50
0
0
90
180
270
365
Age (Days)

Figure 6

Figure 7A

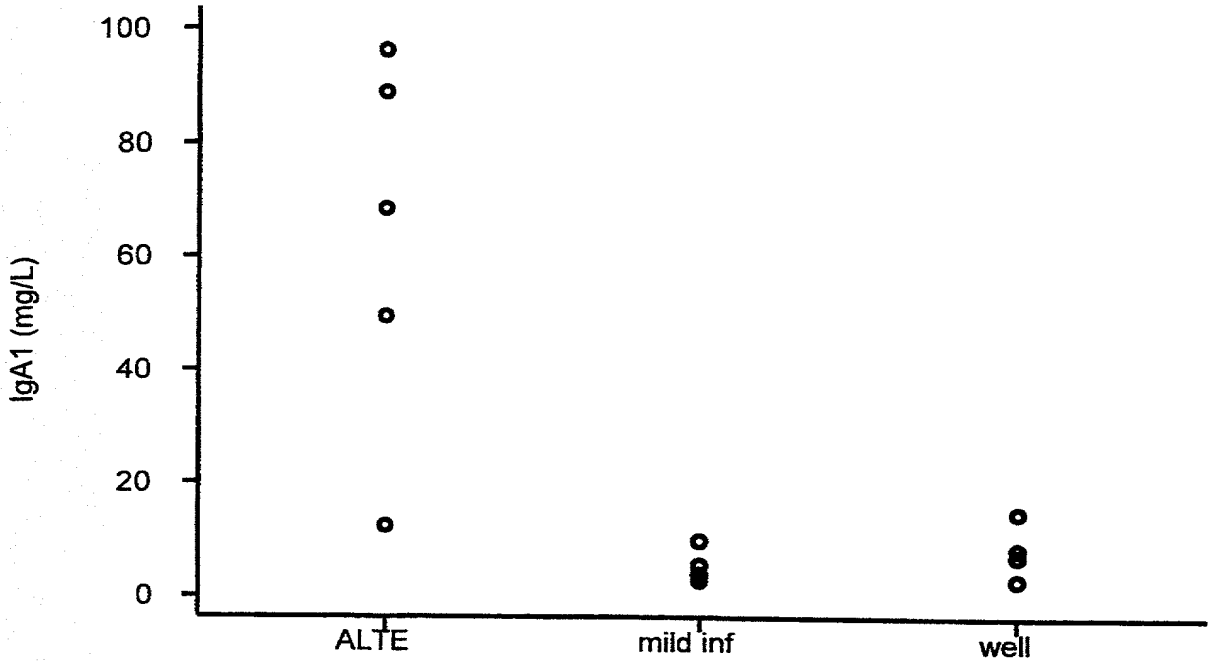


Figure 7B

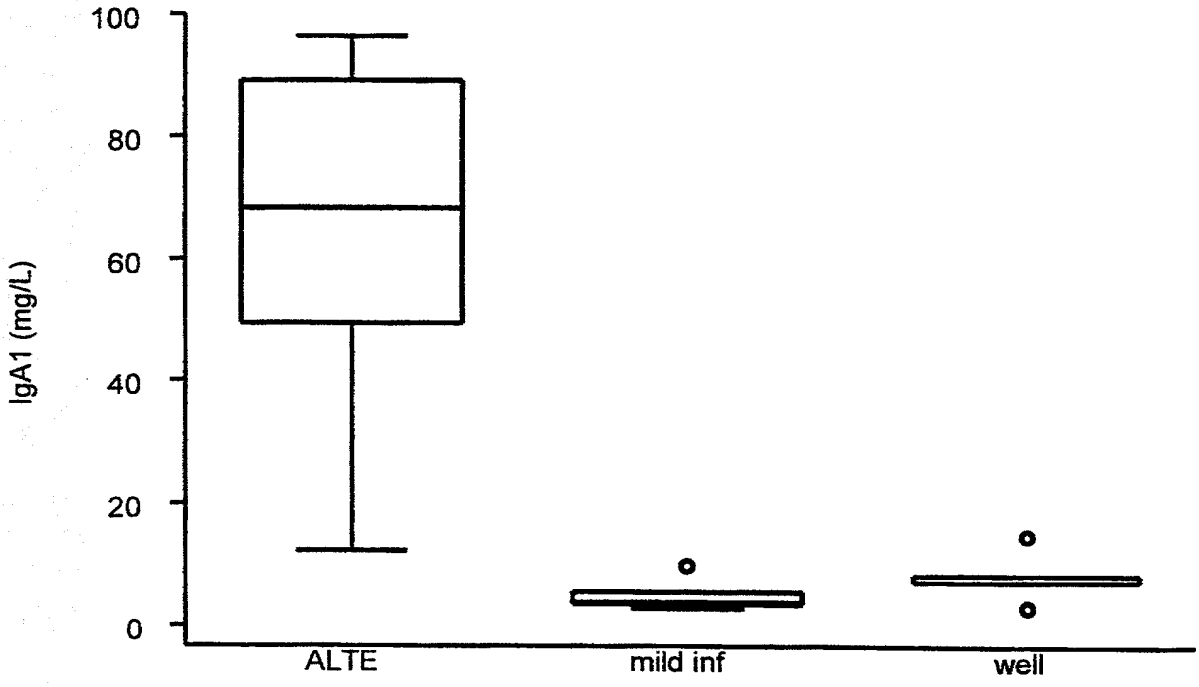
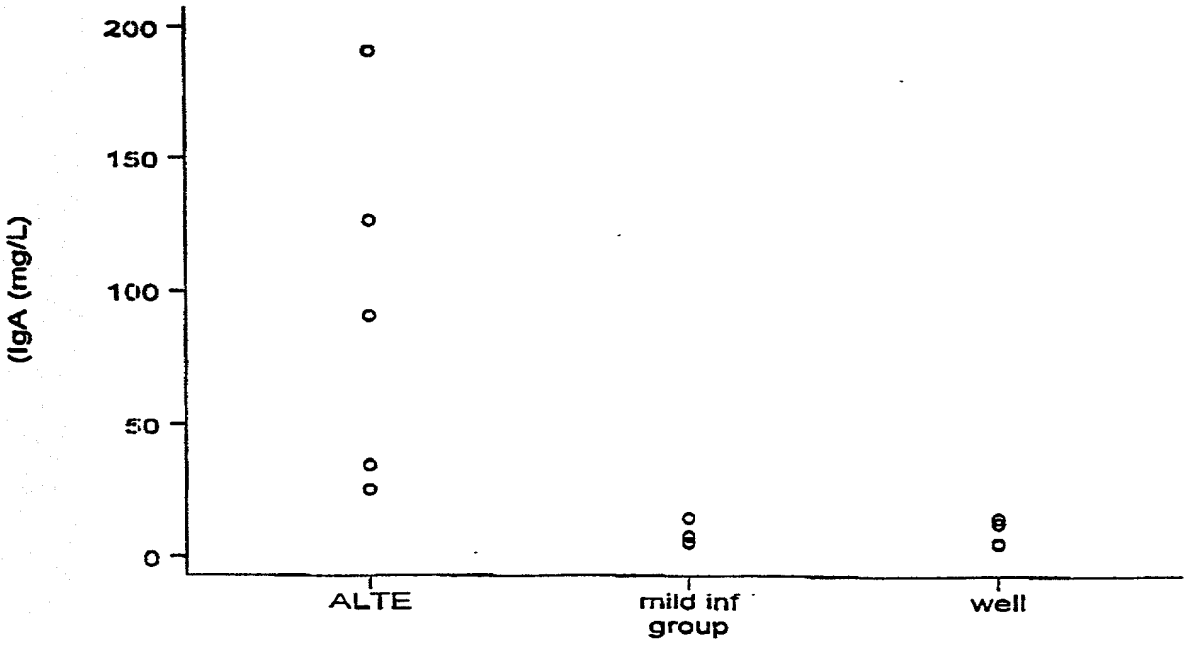


Figure 8



RECEIVED 12 APR 2002

Docket Number BSWV-P01-002

DECLARATION FOR UTILITY PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS

the specification of which was filed on December 7, 2001 as 35 USC 371 Serial No. 10/018,127, on June 7, 2000 as International Patent Application No. PCT/AU00/00643; and was filed on June 7, 1999 as Australian application PQ 0810.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in Title 37, Code of Federal Regulation, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
PCT/AU00/00643 (Number)	International (Country)	07 June 2000 (Day/Month/Year Filed)	☒ Yes ☐ No
PQ 0810 (Number)	Australia (Country)	07 June 1999 (Day/Month/Year Filed)	☒ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States Provisional application(s) listed below.

(Application Number) (Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Number) (Filing Date) (Status: patented, pending, abandoned)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-02

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